Use of a QCM electronic nose to evaluate the aromatic quality in apples

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Abstract The *"Libra Nose"* electronic nose has eight Quartz Microbalance Sensors. When gas molecules are adsorbed onto the quartz crystal surface, the oscillation frequency changes in proportion to the amount of mass. The response of sensors depends on numerous factors that may be difficult to control, such as the temperature and the humidity of the carrier gas. All these factors cause changes in the selectivity of sensors which affect the reproducibility of measurements. In this paper additive and multiplicative corrections are proposed to be applied to the raw sensors's signal in order to eliminate sources of variation. After these corrections, the sensors' response gives useful information to distinguish between batches of apples with different ripeness stage.

Introduction

Quartz crystal microbalances (QCM) are piezoelectric devices; to turn a quartz crystal into a chemical sensor it is necessary to coat it with a layer of a material capable of capturing molecules from the environment. When a mass is adsorbed or placed onto the quartz crystal surface, the oscillation frequency changes, decreasing, in proportion to the amount of mass (Di Natale *et al.* 1997). The response of sensors depends on numerous factors that may be difficult to control, such as the temperature and the humidity of the carrier gas. All these factors cause changes in the selectivity of sensors affecting the reproducibility of measurements (Mielle 1996). Despite previous studies using electronic noses, no QCM calibration statement has been established in relation to the sensitivity needed in the sensors for organoleptic quality measurement in fruit. The objectives are to study the sensor's signal, to identify sources of variation, to define the calibration or correction procedure to remove them and to apply these corrections to measure the ripeness stage of apples.

Materials and methods

The Rome Tor Vergata "Libra nose" electronic nose has 8 Quartz Microbalance Sensors coated with different pyrrolic macrocycle solid-state films, combined for each sensor with different metals (Mn, Ru, Sn, Cr, Co, Cop-OCH3 y Cop-NO2). The sensors are housed in a test chamber having a volume of about 20ml. The users program of "Libra nose" establishes as internal reference the frequency in the starting point (around 20 MHz), that is to say, it obtains a value of relative frequencies subtracting to each measurement its initial value of oscillation frequency after to switching on the equipment. The variable used as sensor response is the increment between the stabilised relative signals, before and after exposure to the gas sample (Δf). One of the problems to evaluate the increment (Δf) is the noise in the base line; to correct it, a software has been developed to smooth and to integrate the signal. The headspace generation system to sample fruit was carried out placing one single and sound fruit inside an hermetic bottle of 0.75 l. On its cover two total-flow cock valves are installed, to make easier and more efficient the transference of headspace to the nose. One hour period for headspace generation is used. When the equilibrium between the gas and liquid phase is achieved, a controlled carrier gas flow of 0.2 l/min, generated by suction with a micro-pump placed inside the nose, carries the effluent towards the sensors. At the same time the second valve is opened entering the carrier gas inside the bottle to avoid vacuum effects. Once the sample achieves a change in the gas sensors oscillation frequency, the sensors chamber must be cleaned. Again is the micro-pump placed inside the nose generates a suction flux of 0.2 l/min and the carrier gas enters in to the nose, removing the gas sample from the sensors' chamber, so that the sensors recover their base line of oscillation frequency.

In this study 2 experiments were carried out to establish the stability of the response of the *Libra Nose* device:

- evaluation of the signal drift between sequences or days of work for a selected reference (1-propanol) throughout a year of measurements (September 99- May 2000, 400 work hours). For headspace generation hermetic bottles of 50 ml were used filled to a 10%, the time of headspace generation was 20 minutes. This study was held simultaneously with a large scale experiment on pears which results are not present in this paper and,
- evaluation of signal drift within a test sequence, measuring the same fruit three times during a day of work: at the beginning, at the middle and at the end of each sequence. This experiment was made using "Fuji" apples and it was repeated during six non consecutive days. The reference (1-propanol) was not used, as might the high concentration of its headspace alter the sensors' signal in the experiment. This study was held simultaneously within a large scale experiment on apples (B.1).

B.1.determination of the influence of shelf life in the aromatic quality of "Fuji" apples. Three times of shelf life (1, 5 and 10 days) were considered after 3 months of cold storage and two different harvest dates (early and late). 60 fruits by harvest date were considered

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(20 fruits x 3 dates). Taking in to account that each day of work one fruit was measured three times as explained paragraph B the total number of samples analysed was equal to 132.

For experiments A the carrier gas used in the transference of headspace or cleaning of sensors′ room was ambient air passing through cartridges of Cl_2Ca to remove the air humidity, and for experiments B and B.1 the headspace transfer and cleaning of sensors′ room were made using synthetic dry air C50 (Carburos Metálicos SA, Madrid) stored in a Teflon bag to avoid overpressures on the micro-pump of the electronic nose.

In this paper, we do not present the electronic nose data crossed and validated with gas chromatography (GC) data, however in all fruit experiments parallel measurements with the electronic nose and with the GC were made by another research team (Post Harvest Department of Lleida University-Udl IRTA, Spain). The dynamic headspace extracted from sound and intact fruits is analysed by GC and mass-spectrometry following the method described by López *et al.* 1998. The results obtained will be presented in later reports.

Results

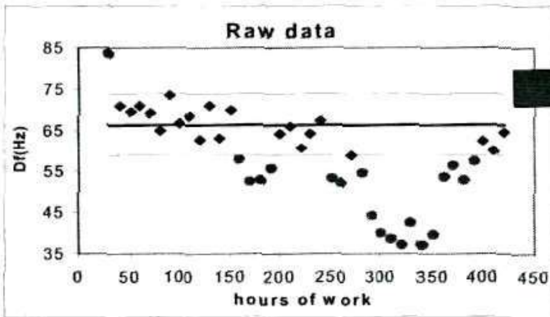
Identification of some sources of variation

The results obtained in experiment A, indicate that in spite of the use of Cl_2Ca cartridge, a cycle of variation is found in the level of response, which is correlated (correlation coefficient, r = 0.66) with changes in the relative humidity (RH) of the ambient, affecting the reproducibility of the measurements between test days. Making the same statistical comparison, with respect to the external temperature, the results show that it does not affect (r = 0.2) the sensors′ response. The temperature that really influences the sensors′ signal is their internal temperature (IT) of work; when the IT reaches or surpasses 37ºC, it produces an obvious instability of the sensors′ signal along a sequence of measurements.

The lack of reproducibility between sequences or test days (effect of RH) and within sequences or test day (effect of IT), forces to carry out a set of calibrations or corrections of the sensors′ signal. The mentioned sources of variation are not, probably, the only ones and at this stage they are subjected to research.

Additive corrections

As it can be seen on Figure 1, the data corresponding to the average of the daily response of all sensors for 1-propanol (experiment A), show a low reproducibility. An additive correction (see Fig. 2) is proposed to improve the reproducibility level, estimated as standard deviation (STD) of daily average, from ±11.23 Hz to ±3.26 Hz.



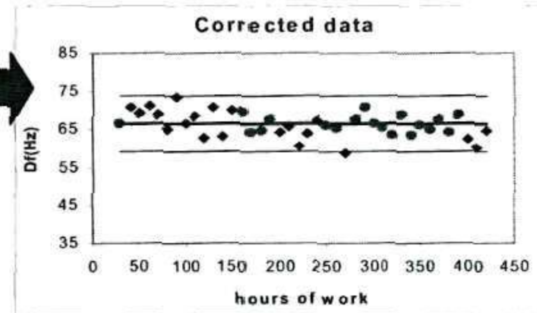


Fig. 1. Average response of all eight sensors of the electronic nose for 1-Propanol

Fig. 2. Average values after additive correction

Multiplicative correction

Figure 3 shows the result obtained in experiment B, indicating that there is an increase of the average response for all sensors (Δf) from the beginning of the sequence (0 min), along the time (180 min), reaching its maximum value at the end of a day of work (400 min). In Figure 3 each dot of the plot is the average of 6 repetitions. It can be observed that the increase of the response is not linear from 0 to 400 minutes. It is possible to distinguish two section defined by two different slopes or drifts. The drift (d) has been computed as follows:

$$d_{0\text{-}400}(Hz/min) = \frac{\Delta f_{400} - \Delta f_0}{400}; \quad d_{0\text{-}180}(Hz/min) = \frac{\Delta f_{180} - \Delta f_0}{180}; \quad d_{180-400}(Hz/min) = \frac{\Delta f_{400} - \Delta f_{180}}{220}$$

To be sure that these drifts are statistically significant it is necessary to apply some steps analysis:

first step: when $d_{0\text{-}400}$ minus 1.96 standard error (SE) is positive, the global drift is significantly different from 0 (horizontal) and therefore there is a need for correction,

second step: $d_{0\text{-}180}$ and $d_{180\text{-}400}$ can be compared by means of a one-way analysis of variance (ANOVA); when the p value is below 0.05 both drifts are significantly different and therefore different correction has to be applied for each period, and

third step: it consists of comparing each of the partial drifts ($d_{0\text{-}180}$ and $d_{180\text{-}400}$) with respect to no drift as mentioned for step 1.

From this analysis, it can be that a multiplicative correction (extracted drift correction) is necessary only for the first period ($d_{0\text{-}180}$). Applying multiplicative correction the average variability of data (estimated as STD) decreases from ±24.1 Hz to ±6.2 Hz.

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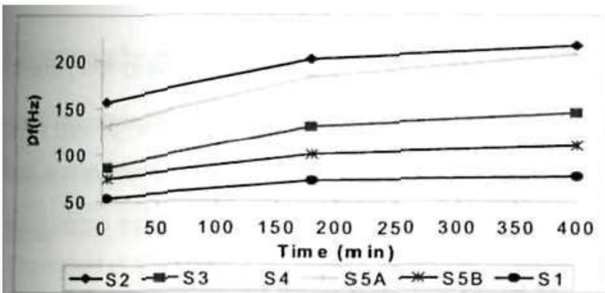
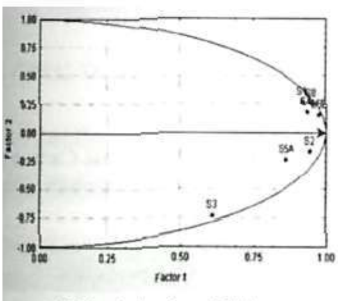


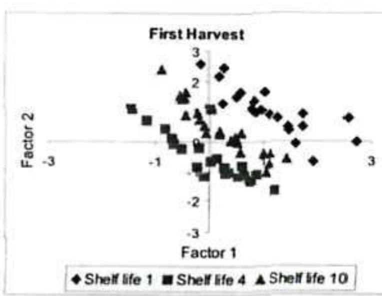
Fig. 3. Average evolution (n=6) of Δf for some sensors, with respect to time, for a same fruit and day of work

Application of additive & multiplicative correction on fruit data

Applying the corrections described in paragraphs 3.2 and 3.3 on data obtained from experiment B.1. using *Fuji* apples, a PCA has carried out which results are explained on Figure 4. This PCA analysis shows that clearly all sensors are giving a very similar information, defining the Factor1 of the PCA; just the contribution of sensor 3 makes a difference, defining a second factor in the PCA. Along Factor 1 of PCA, it is possible to distinguish between the three times of shelf life considered, mainly for early harvest (Fig. 4, right).



PCA plot of variables



PCA scores plot: Early harvest

Fig. 4. PCA for the experiment on Fuji apples (n=132). Shelf life evolution has been evaluated for two different harvest dates (early and late). Additive and drift correction are used

The evolution from a physiological point of view of *Fuji* apples during the ripeness process, is similar to a non-climacteric fruit, carrying out very few changes along that process; this may be the reason that explains the lack of segregation by shelf life for the fruit batches corresponding to second harvest. Along the axis defined by sensor 3 exists a variability inside each group that at this stage we can not explain. Sensor 3 is reacting to an effect unknown for us and thus not controlled.

Conclusions

- This work shows that the stability and the reproducibility of sensors´ response is low, due to external and internal factors. The relative humidity of the ambient air and the internal temperature of work for the sensors are an example but the majority of them are unknown and thus not controlled, introducing sources of variation, forcing to carry out additive corrections between sequences and multiplicative corrections within each sequence.
- The application of additive correction improves the reproducibility level, estimated as standard deviation of daily average, using 1-propanol as reference product.
- The application of multiplicative correction allows to remove the drift or the increment of signal along a test day, decreasing the average variability of data.
- The application of additive and multiplicative correction allows in *Fuji* apples to detect differences in the aromatic production by the fruit along 1, 5 and 10 days of shelf life.

Acknowledgment

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